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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C07D 305/14 — A1

(11) International Publication Number: WO 92/18492

(43) International Publication Date: 29 October 1992 (29.10.92)

(21) International Application Number: PCT/US92/03088

(22) International Filing Date: 14 April 1992 (14.04.92)

(30) Priority data:
690,805 19 April 1991 (19.04.91) US

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(81) Designated States: AT (European patent), AU, BE (European patent), BG, CA, CH (European patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LU (European patent), MC (European patent), NL (European patent), NO, PL, RO, RU, SE (European patent).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHODS AND COMPOSITIONS FOR ISOLATING TAXANES

(57) Abstract

Methods of obtaining renewable sources of taxanes including taxol are provided. Compositions comprising taxanes which are useful as source materials for the further purification of taxanes are also disclosed. Specifically, a method of drying plant matter to preserve their taxane content and facilitate their extraction is disclosed. In addition, methods of extracting and purifying taxol and other taxanes from ornamental cultivars using a series of organic and aqueous solvents and normal phase chromatography columns are also disclosed.

We claim:

1. A process for obtaining taxanes from plants said process comprising providing a renewable source of plant matter from one or more cultivars of ornamental Taxus plants wher[redacted] wherein a substantial amount of foliage is attached to the stems, and [redacted] obtain a taxane-rich product.

2. The process according to claim 1 wherein the renewable source of plant matter is a cultivar of the Taxus genus selected from the group consisting of T. X media 'Henryi', T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Fairview', T. cuspidata 'Brevifolia', T. cuspidata, and T. cuspidata 'Spreader'.

3. The process according to claim 2 wherein the renewable source of plant matter is a cultivar of the Taxus genus selected from the group consisting of T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. cuspidata 'Brevifolia', and T. cuspidata.

4. The process according to claim 2 wherein the renewable source of plant matter is a cultivar of the Taxus genus selected from the group consisting of T. cuspidata, T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Tauntonii'; T. X media 'Nigra', T. X media 'Dark Green Spreader', and T. X media 'Hicksii'.

5. The process according to claim 1 wherein the plant matter is subjected to a drying step prior to solvent treatment.

6. The process according to claim 5 wherein the drying step is not conducted under unobstructed direct sunlight.

7. The process according to claim 1 wherein the plant matter is ground prior to solvent treatment.

8. The process according to claim 1 wherein the plant matter that is subjected to solvent treatment retains a substantial portion of its original volatile contents.

9. A taxane-rich composition prepared by providing a renewable source of plant matter from one or more cultivars of ornamental Taxus plants wherein said plant matter is in the form of an intact clipping, wherein a substantial amount of foliage is attached to the stems, and treating the plant matter with one or more solvents to obtain said taxane-rich product.

10. The composition according to claim 9 wherein the renewable source of plant matter is a cultivar of the Taxus genus selected from a variety of Taxus selected from the group consisting of T. X media 'Henryi', T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Fairview', T. cuspidata 'Brevifolia', T. cuspidata, and T. cuspidata 'Spreader'.

11. The composition according to claim 9 wherein the renewable source of the plant matter is a cultivar of the Taxus genus selected from the group consisting of T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media

'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. cuspidata 'Brevifolia', and T. cuspidata.

12. The composition according to claim 9 wherein the renewable source of plant matter is a cultivar of the Taxus genus selected from the group consisting of T. cuspidata, T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Tauntonii', T. X media 'Nigra', T. X media 'Dark Green Spreader', and T. X media 'Hicksii'.

13. The composition according to claim 9 wherein the plant matter is ground prior to solvent treatment.

14. The composition according to claim 9 wherein the plant matter that is subjected to solvent treatment retains a substantial portion of its original volatile contents.

15. The composition according to claim 9 wherein the plant matter is subjected to a drying step prior to solvent treatment.

16. The compositions according to claim 15 wherein the drying step is not conducted under unobstructed direct sunlight.

17. A process for preparing a plant material which can be used as a source for taxanes, said process comprising providing intact clippings, wherein a substantial amount of foliage is attached to the stems of taxane-containing plant matter, drying the intact clippings to reduce its volatile content to produce substantially dry clippings, and recovering said substantially dry clippings.

18. The process for preparing a plant material according to claim 17 wherein the drying step is conducted at a temperature of less than about 70°C.

19. The process for preparing a plant material according to claim 17 wherein the drying step is conducted at a temperature of between about 20°C and about 65°C.

20. The process for preparing a plant material according to claim 17 wherein the drying step is not conducted under unobstructed direct sunlight.

21. The process for preparing a plant material according to claim 20 wherein between about 40% and about 80% of visible sunlight is prevented from reaching the plant matter during the drying step.

22. The process for preparing a plant material according to claim 17 wherein at least a 25% reduction in weight of the plant matter is obtained during the drying step.

23. The process for preparing a plant material according to claim 22 wherein the plant matter is dried until between about a 40% and about a 70% weight loss of the plant matter is obtained during the drying step.

24. The process for preparing a plant material according to claim 17 wherein the plant matter is selected from one or more cultivars of ornamental Taxus plants.

25. The process for preparing a plant material according to claim 24 wherein the plant matter is selected from one or more Taxus species selected from the group consisting of T. X media 'Henryi', T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Fairview', T. cuspidata 'Brevifolia', T. cuspidata, and T. cuspidata 'Spreader'.

26. The process for preparing a plant material according to claim 25 wherein the plant matter is from one or more Taxus species selected from the group consisting of T. cuspidata, T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Dark Green Spreader', and T. X media 'Hicksii'.

27. The process for preparing a plant material according to claim 26 wherein the plant matter is subjected to a drying step at a temperature of less than about 70°C and which drying step is not conducted under unobstructed direct sunlight.

28. A plant material which can be used as a source for taxanes, said material prepared according to the process comprising providing intact clippings, wherein a substantial amount of foliage is attached to the stems of taxane-containing plant matter, drying the intact clippings to reduce the volatile component to produce substantially dry clippings and recovering said substantially dry clippings.

29. The plant material according to claim 28 wherein the drying step is conducted at a temperature of less than about 70°C.

30. The plant material according to claim 29 wherein the drying step is conducted at a temperature of between about 20°C and about 65°C.

31. The plant material according to claim 28 wherein the drying step is not conducted under unobstructed direct sunlight.

32. The plant material according to claim 31 wherein between about 40% and about 80% of visible sunlight is prevented from reaching the plant matter during the drying step.

33. The plant material according to claim 28 wherein at least a 25% reduction in weight of the plant matter is obtained during the drying step.

34. The plant material according to claim 28 wherein the plant matter is dried until between about a 40% and about a 70% weight loss of the plant matter is obtained during the drying step.

35. The plant material according to claim 28 wherein the plant matter is from one or more species of cultivated ornamental Taxus.

36. The plant material according to claim 28 wherein the plant matter is selected from one or more Taxus species selected from the group consisting of T. X media 'Henryi', T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Fairview', T. cuspidata 'Brevifolia', T. cuspidata, and T. cuspidata 'Spreader'.

37. The plant material according to claim 36 wherein the plant matter is selected from the group consisting of T. cuspidata, T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Dark Green Spreader', and T. X media 'Hicksii'.

38. The plant material according to claim 37 wherein the plant matter is subjected to a drying step at a temperature of less than about 70°C and which drying step is not conducted under unobstructed direct sunlight.

39. A process for obtaining a crude taxane mixture from taxane-containing plant matter, said process comprising:

an initial solvent treatment comprising the steps of:

A. extracting taxanes from plant matter by contacting the [redacted] with an organic solvent and obtaining a substantially solvent-free first residue rich in taxanes;

B. partitioning the first residue between an organic solvent capable of dissolving the taxanes and an aqueous solvent which does not dissolve significant quantities of taxanes to produce an organic phase and an aqueous phase;

C. separating the organic phase from the aqueous phase and recovering the organic phase substantially free of the aqueous phase;

D. forming a second residue from the second organic phase;

subjecting said second residue to a finishing treatment selected from either

E. dissolving the second residue in a solvent which allows for the reversible attachment of the taxanes to a solid support,

introducing a solid support into the solvent comprising the dissolved second residue and allowing the taxanes of the second residue to attach to said solid support,

separating said solid support from the solvent,

sequentially contacting the solid support with attached taxanes with a series of solvents which solvents have differing taxane eluting properties, and

eluting a taxane-rich fraction from the solid support as the crude taxane mixture, or

F. partitioning the second residue between an aqueous mixture and a nonpolar nonmiscible organic solvent, wherein the aqueous mixture comprises a single

phase of water and a polar organic solvent miscible with water which polar organic solvent is present in said mixture in an amount sufficient to allow the taxanes present in the second residue to enter the aqueous mixture, and

recovering the crude taxane mixture from the second aqueous mixture.

40. The process according to claim 39 wherein any of steps A, B, C, D, E or F are repeated one or more times.

41. The process according to claim 39 wherein the plant matter in step A is contacted with an organic solvent, selected from the group consisting of ethanol, acetone, ethyl acetate, methylene chloride, chloroform, carbon tetrachloride, methyl ethyl ketone, methyl isobutyl ketone, methyl t-butyl ether, methanol or mixtures thereof.

42. The process according to claim 41 wherein the plant matter in step A is contacted with an organic solvent selected from the group consisting of acetone, ethanol and ethyl acetate.

43. The process according to claim 42 wherein the plant matter in step A is contacted with ethanol.

44. The process according to claim 39 wherein contact of the plant matter with the solvent in step A is promoted by a method selected from the group consisting of soaking, percolation, soxhlet extraction, agitating, or a combination thereof.

45. The process according to claim 44 wherein contact of the plant matter in step A with the organic solvent is promoted by shaking.

46. The process according to claim 45 wherein contact of the plant matter in step A with the

organic solvent is promoted by soaking and agitating the plant matter in the organic solvent.

47. The process according to claim 39 wherein prior to contacting the plant matter in step A with the organic solvent, the plant matter is defatted with a solvent selected from the group consisting of hexane, hexanes, pentane, petroleum ether, isooctane, or mixtures thereof.

48. The process according to claim 39 wherein the organic solvent of step B is selected from the group consisting of methylene chloride and ethyl acetate.

49. The process according to claim 48 wherein the organic solvent of step B is ethyl acetate.

50. The process according to claim 39 wherein the aqueous solvent of step B is water.

51. The process according to claim 39 wherein the organic solvent of step C is treated with a drying agent or process following the separation of the organic solvent from the aqueous solvent.

52. The process according to claim 51 wherein the drying agent is selected from the group consisting of anhydrous magnesium sulphate, anhydrous sodium sulphate, 4Å molecular sieves, calcium chloride or mixtures thereof and the drying process comprises cooling the organic solvent to a temperature at or below that at which water freezes and separating the liquid organic solvent from the frozen water.

53. The process according to claim 52, wherein the drying agent is anhydrous sodium sulphate.

54. The process according to claim 39 wherein the plant matter is selected from one or more cultivars of ornamental Taxus species.

55. The process according to claim 54 wherein the plant matter is obtained from a cultivar of

the Taxus genus selected from the group consisting of T. X media 'Henryi', T. X media 'Densiformis', T. X media 'Hicksii', T. X media 'Dark Green Spreader', T. X media 'Runyan', T. X media Brownii', T. X media 'Wardii', T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Fairview', T. cuspidata 'Brevifolia', T. cuspidata, and T. cuspidata 'Spreader'.

56. The process according to claim 39 wherein the plant matter to be extracted is subjected to a drying step prior to extraction.

57. The process according to claim 56 wherein the plant matter subjected to the drying step is provided as intact clippings wherein a substantial amount of foliage is attached to the stems.

58. The process according to claim 57 wherein the drying step is conducted at a temperature of less than about 70°C.

59. The process according to claim 58 wherein the drying step is conducted at a temperature of between about 20°C and about 65°C.

60. The process according to claim 56 wherein the drying step is not conducted under unobstructed direct sunlight.

61. The process according to claim 60 wherein between about 40% and about 80% of visible sunlight is prevented from reaching the plant matter during the drying step.

62. The process according to claim 56 wherein at least a 25% reduction in weight of the plant matter is obtained during the drying step.

63. The process according to claim 62 wherein the plant matter is dried until between about a 40% and about a 70% weight loss of the plant matter is obtained during the drying step.

64. The process according to claim 39 wherein the crude taxane mixture comprises the taxanes selected from the group consisting of taxol, cephalomannine, desacetyl-cephalomannine, baccatin III, 10-desacetyl baccatin III and 10-desacetyltaxol.

65. The process according to claim 39 wherein the second residue is subjected to the finishing treatment of step E.

66. The process for obtaining a crude taxane mixture according to claim 65 wherein the solvent of step E for dissolving the second residue of step D is a mixture of ethyl acetate and methanol.

67. The process for obtaining a crude taxane mixture according to claim 65 wherein the solvent of step E for dissolving the second residue of step D is ethyl acetate.

68. The process for obtaining a crude taxane mixture according to claim 65 wherein the solid support of step E is celite.

69. The process for obtaining a crude taxane mixture according to claim 65 wherein taxanes are reversibly attached to the solid support of step E by adding said solid support to said solvent of step E in which the second residue has been dissolved and causing said solvent to evaporate resulting in components of said second residue attaching to said solid support.

70. The process for obtaining a crude taxane mixture according to claim 65 wherein the solid support of step E to which components of the second residue have been attached is first eluted with a solvent which does not remove substantial amounts of taxane from the solid support, followed by a second elution with a solvent which is more polar than the solvent used for the first elution, which more polar solvent elutes a taxane-rich fraction from the solid support as the crude taxane mixture.

71. The process for obtaining a crude taxane mixture according to claim 70 wherein the solvent used for the first elution is hexane.

72. The process for obtaining a crude taxane mixture according to claim 70 wherein the solvent used to elute the taxanes from the solid support is selected from the group consisting of ethyl acetate and methylene chloride.

73. The process for obtaining a crude taxane mixture according to claim 70 wherein hexane is used for the first elution and ethyl acetate is used to elute the taxanes.

74. The process according to claim 39 wherein the second residue is subjected to the finishing treatment of step F.

75. The process according to claim 74 wherein the aqueous mixture of step F comprises more

than about 50% polar organic solvent and the balance water.

76. The process according to claim 75 wherein the aqueous mixture of step F comprises about 9 parts polar organic solvent and about 1 part water.

77. The process according to claim 74 wherein the polar organic solvent of the aqueous mixture of step F is selected from the group consisting of acetonitrile and methanol.

78. The process according to claim 74 wherein the polar organic solvent of the aqueous mixture of step F is methanol.

79. The process according to claim 74 wherein the aqueous mixture of step F comprises more than about 50% methanol and the balance water.

80. The process according to claim 79 wherein the aqueous mixture of step F comprises about 9 parts methanol and about 1 part water.

81. The process according to claim 74 wherein the organic solvent of step F is selected from the group consisting of hexane, pentane, petroleum ether, heptane, iso-octane and mixtures thereof.

82. The process according to claim 81 wherein the organic solvent of step F is hexane.

83. The process according to claim 74 wherein the second residue is partitioned between a

nonpolar nonmiscible organic solvent comprising hexane and an aqueous mixture comprising methanol and water.

84. The process according to claim 74 wherein the second residue is partitioned between a greater volume of nonpolar nonmiscible organic solvent than aqueous mixture.

85. The process according to claim 84 wherein the second residue is partitioned between about 1 part second aqueous mixture and about 2 parts nonpolar nonmiscible organic solvent.

86. The process according to claim 74 wherein the crude taxane mixture comprises the taxanes selected from the group consisting of taxol, cephalomannine, desacetyl-cephalomannine, baccatin III, 10-desacetyl baccatin III and 10-desacetyltaxol.

87. A crude taxane mixture from plant matter containing taxanes prepared according to the steps comprising:

A. extracting taxanes from plant matter by contacting the plant matter with an organic solvent and obtaining a substantially solvent-free first residue rich in taxanes;

B. partitioning the first residue between an organic solvent capable of dissolving the taxanes and an aqueous solvent which does not dissolve significant quantities of taxanes to produce an organic phase and an aqueous phase;

C. separating the organic phase from the aqueous phase and recovering the organic phase substantially free of the aqueous phase;

D. forming a second residue from the second organic phase;

subjecting said organic phase to a finishing solvent treatment selected from either

E. dissolving the second residue in a solvent which allows for the reversible attachment of the taxanes unto a solid support,

introducing a solid support into the solvent comprising the dissolved second residue and allowing the taxanes of the second residue to attach to said solid support,

separating said solid support from the solvent,

sequentially contacting the coated solid support with attached taxanes with a series of solvents which solvents have differing taxane eluting properties, and

eluting a taxane-rich fraction from the solid support as the crude taxane mixture, or

F. partitioning the second residue between an aqueous mixture and a nonpolar nonmiscible organic solvent, wherein the aqueous mixture comprises a single phase of water and a polar organic solvent miscible with water which polar organic solvent is present in

said mixture in an amount sufficient to allow the taxanes present in the second residue to enter the aqueous mixture, and

recovering the crude taxane mixture from the aqueous mixture.

88. The crude taxane mixture according to claim 87 wherein the plant matter is provided from one or more species of cultivars of the ornamental Taxus genus.

89. The crude taxane mixture according to claim 87 wherein the plant matter to be extracted is provided as an intact clipping wherein a substantial amount of foliage is attached to stems and subjected to a drying step prior to extraction.

90. The crude taxane mixture according to claim 87 wherein the second residue is subjected to the finishing treatment of step E.

91. The crude taxane mixture according to claim 87 wherein the second residue is subjected to the finishing treatment of step F.

92. The crude taxane mixture according to claim 91 wherein the aqueous mixture of step F comprises more than about 50% polar organic solvent and the balance water.

93. The crude taxane mixture according to claim 92 wherein the aqueous mixture of step F

comprises about 9 parts polar organic solvent and about 1 part water.

94. The crude taxane mixture according to claim 91 wherein the polar organic solvent of the aqueous mixture of step F is selected from the group consisting of acetonitrile and methanol.

95. The crude taxane mixture according to claim 94 wherein the polar organic solvent of the aqueous mixture of step F is methanol.

96. The crude taxane mixture according to claim 91 wherein the aqueous mixture of step F comprises more than about 50% methanol and the balance water.

97. The crude taxane mixture according to claim 96 wherein the aqueous mixture of step F comprises about 9 parts methanol and about 1 part water.

98. The crude taxane mixture according to claim 91 wherein the organic solvent of step F is selected from the group consisting of hexane, pentane, petroleum ether, heptane, iso-octane and mixtures thereof.

99. The crude taxane mixture according to claim 98 wherein the organic solvent of step F is hexane.

100. The crude taxane mixture according to claim 91 wherein the second residue is partitioned

between a nonpolar nonmiscible organic solvent comprising hexane and an aqueous mixture comprising methanol and water.

101. The crude taxane mixture according to claim 91 wherein the second residue is partitioned between a greater volume of nonpolar nonmiscible organic solvent than aqueous mixture.

102. The crude taxane mixture according to claim 101 wherein the second residue is partitioned between about 1 part second aqueous mixture and about 2 parts nonpolar nonmiscible organic solvent.

103. The crude taxane mixture according to claim 87 wherein the crude taxane mixture comprises the taxanes selected from the group consisting of taxol, cephalomannine, desacetyl-cephalomannine, baccatin III, 10-desacetyl baccatin III and 10-desacetyltaxol.

104. A process for separating two or more taxanes including taxol and cephalomannine comprising the steps of:

providing a mixture comprising two or more taxanes in a solvent suitable for loading onto a normal phase chromatography column;

loading the taxane comprising mixture onto a normal phase chromatography column packed with a solid support in a solvent suitable as a mobile phase;

separating the taxanes by eluting the normal phase chromatography column with a mobile phase having

a sufficient polarity to separately elute taxol and cephalomannine.

105. The process according to claim 104 wherein the mobile phase is a mixture of ethyl acetate and methylene chloride.

106. The process according to claim 105 wherein the mixture of taxanes is loaded onto the chromatography column in a mixture of about 20% ethyl acetate in methylene chloride.

107. The process according to claim 104 wherein substantially pure taxol is collected from fractions eluted with about 45% ethyl acetate and about 55% methylene chloride.

108. The process according to claim 104 wherein substantially pure cephalomannine is collected from fractions eluted with about 50% ethyl acetate and about 50% methylene chloride.

109. A process for obtaining and separating the taxanes taxol and cephalomannine from taxane-containing plant matter comprising the steps of:

providing intact clippings wherein a substantial amount of foliage is attached to the stems of taxane-containing plant matter, drying the intact clippings to reduce its volatile content to produce substantially dry clippings and recovering said substantially dry clippings,

extracting taxanes from said substantially dry clippings according to the following steps comprising:

A. contacting the plant matter with an organic solvent and obtaining a substantially solvent-free first residue rich in taxanes;

B. partitioning the first residue between an organic solvent capable of dissolving the taxanes and an aqueous solvent which does not dissolve significant quantities of taxanes to produce an organic phase and an aqueous phase;

C. separating the organic phase from the aqueous phase and recovering the organic phase substantially free of the aqueous phase;

D. forming a second residue from the second organic phase;

subjecting said organic phase to a finishing treatment selected from either

E. dissolving the second residue in a solvent which allows for the reversible attachment of the taxanes unto a solid support,

introducing a solid support into the solvent comprising the dissolved second residue and allowing the taxanes of the second residue to attach to said solid support,

separating said solid support from the solvent,

sequentially contacting the solid support with attached taxanes with a series of solvents which solvents have differing taxane eluting properties, and

eluting a taxane-rich fraction from the solid support as the crude taxane mixture, or

F. partitioning the second residue between an aqueous mixture and a nonpolar nonmiscible organic solvent, wherein the aqueous mixture comprises a single phase of water and a polar organic solvent miscible with water which polar organic solvent is present in said mixture in an amount sufficient to allow the taxanes present in the second residue to enter the aqueous mixture, and

recovering the crude taxane mixture from the second aqueous mixture,

providing the crude taxane mixture obtained from either treatments E or F, said crude taxane mixture comprising two or more taxanes, in a solvent suitable for loading onto a normal phase chromatography column;

loading the taxane comprising mixture onto a normal phase chromatography column packed with a solid support in a solvent suitable as a mobile phase;

separating the taxanes by eluting the normal phase chromatography column with a mobile phase having a sufficient polarity to separately elute taxol and cephalomannine.

110. The process according to claim 109 wherein the drying step is conducted at a temperature of less than about 70°C.

111. The process according to claim 110 wherein the drying step is conducted at a temperature of between about 20°C and 65°C.

112. The process according to claim 109 wherein the drying step is not conducted under unobstructed direct sunlight.

113. The process according to claim 112 wherein between about 40% and about 80% of visible sunlight is prevented from reaching the plant matter during the drying step.

114. The process according to claim 109 wherein at least a 25% reduction in weight of the plant matter is obtained during the drying step.

115. The process according to claim 114 wherein the plant matter is dried until between about a 40% and about a 70% weight loss of the plant matter is obtained during the drying step.

116. The process according to claim 109 wherein the plant matter is selected from the group consisting of T. cuspidata, T. X media 'Halloran', T. X media 'Hatfield', T. X media 'Nigra', T. X media 'Tauntonii', T. X media 'Dark Green Spreader', and T. X media 'Hicksii'.

117. The process according to claim 109 wherein the second residue is subjected to the finishing treatment of step F.

118. The process according to claim 117 wherein the aqueous mixture of step F comprises more than about 50% polar organic solvent and the balance water.

119. The process according to claim 118 wherein the aqueous mixture of step F comprises about 9 parts polar organic solvent and about 1 part water.

120. The process according to claim 117 wherein the polar organic solvent of the aqueous mixture of step F is selected from the group consisting of acetonitrile and methanol.

121. The process according to claim 120 wherein the polar organic solvent of the aqueous mixture of step F is methanol.

122. The process according to claim 117 wherein the aqueous mixture of step F comprises more than about 50% methanol and the balance water.

123. The process according to claim 122 wherein the aqueous mixture of step F comprises about 9 parts methanol and about 1 part water.

124. The process according to claim 117 wherein the organic solvent of step F is selected from the group consisting of hexane, pentane, petroleum ether, heptane, iso-octane and mixtures thereof.

125. The process according to claim 124 wherein the organic solvent of step F is hexane.

126. The crude taxane mixture according to claim 117 wherein the second residue is partitioned between a nonpolar nonmiscible organic solvent comprising hexane and an aqueous mixture comprising methanol and water.

127. The crude taxane mixture according to claim 117 wherein the second residue is partitioned between a greater volume of nonpolar nonmiscible organic solvent than aqueous mixture.

128. The process according to claim 127 wherein the second residue is partitioned between about 1 part second aqueous mixture and about 2 parts nonpolar nonmiscible organic solvent.

129. The process according to claim 117 wherein the crude taxane mixture comprises the taxanes selected from the group consisting of taxol, cephalomannine, desacetyl-cephalomannine, baccatin III, 10-desacetyl baccatin III and 10-desacetyltaxol.

130. The process according to claim 129 wherein the taxane obtained and separated is taxol.

131. The process according to claim 40 wherein any of steps A, B, C. D, E or F are repeated one, two, three or four times.

132. The process according to claim 104 wherein any of the steps for separating two or more taxanes are repeated one or more times.

133. The process according to claim 132 wherein any of the steps are repeated one, two, three or four times.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 92/03088

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]
According to International Patent Classification (IPC) or to both National Classification and IPC
Int.Cl. 5 C07D305/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07D ; C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category[9]	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
Y,P	JOURNAL OF LIQUID CHROMATOGRAPHY vol. 15, no. 4, 1992, pages 697 - 706; R. VANHAELEN-FASTRE ET AL.: 'HIGH-SPEED COUNTERCURENT CHROMATOGRAPHY SEPARATION OF TAXOL AND RELATED DITERPENOIDS FROM TAXUS BACCATA' THE WHOLE ARTICLE ---	1
Y,P	JOURNAL OF CHROMATOGRAPHY. vol. 587, 1991, AMSTERDAM NL pages 300 - 305; S.D. HARVEY ET AL.: 'SEPARATION OF TAXOL FROM RELATED TAXANES IN TAXUS BREVIFOLIA EXTRACTS BY ISOCRATIC ELUTION REVERSED-PHASE MICROCOLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY' THE WHOLE ATICLE --- -/—	1

* Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
25 AUGUST 1992	01. 09. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	REMPP G.L.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	PLANTA MED. vol. 56, 1990, NEW YORK pages 293 - 294; ZONGPING ZHANG ET AL.: 'NEW TAXANES FROM TAXUS CHINENSIS' THE WHOLE ARTICLE ---	1